

# RECIPROCAL COURTSHIP EXPERIMENTS BETWEEN ALLOPATRIC POPULATIONS OF *Theridion grallator*: ASYMMETRICAL ISOLATION AND INCIPIENT SPECIATION DEPENDS ON HABITAT HETEROGENEITY

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## ABSTRACT:

Allopatric populations of *Theridion grallator* Simon show morphological, demographic, ecological and behavioral differences that indicate divergence. Differences in courtship behavior suggest intrinsic pre-zygotic isolating mechanisms, and reciprocal cross-mating experiments were conducted between different study sites on the islands of Hawai'i and Maui. All inter-island attempts failed to culminate in copulation, and intra-island attempts showed either incomplete and asymmetrical ethological barriers (Hawai'i sites), or successful courtships to copulation (Maui sites). The results justify the naming of certain geographical taxa of *Theridion grallator* as separate species. Asymmetries are discussed in terms of recent theory regarding asymmetrical courtship barriers and the direction of evolution. Habitat heterogeneity is invoked to explain the maintenance of deme structure on Maui relative to Hawai'i.

## INTRODUCTION

Speciation involves the development of reproductive isolating mechanisms that prevent gene flow between populations (e.g., Mayr, 1963; Dobzhansky 1940; Zimmerman, 1948; White, 1968). In many animals, reproductive isolation occurs at the level of behavioral differences in courtship that allow for the identification of conspecifics and rejection of allospecific individuals (e.g., Mayr, 1963; Bastock, 1967). There are few opportunities to study divergence of courtship behaviors and to test alternate hypotheses regarding the factors involved in incipient speciation (e.g., see Ohta, 1978).

*Theridion grallator* (Simon) is a spider endemic to the Hawaiian islands of Hawai'i, Maui, Moloka'i and O'ahu in middle altitude forest habitats. Allopatric populations show differences along morphological, demographic, ecological and behavioral axes (Gon, in press; Gon, in prep.). Observable differences in courtship behavior have been quantified (Gon and Reese, in prep.), and suggest that ethological isolating mechanisms may have arisen. This paper presents the results of a test of reproductive isolation via reciprocal cross mating experiments between chosen populations of *Theridion grallator*. It compares inter-island and intra-island barriers to gene flow, provides the justification for taxonomic revisions of the species, and discusses inferences regarding asymmetries in courtship barriers and the direction of evolution.

## METHODS

Three permanent study sites and one additional sample site served as sources for cross population mating attempts. The locations of the sites are presented in Figure 1. The sites were chosen for their separation in space and for the presence of recognizable extrinsic barriers to gene flow. On the island of Hawai'i, the Kahauale'a and Manukā sites are separated by 77 km of linear distance, and are on opposite flanks of the Mauna Loa shield volcano (4169 m in height.). For the island of Maui, the Kīpahulu and Makawao sites are separated by 22 km linear distance and are on opposite flanks of the East Maui shield volcano (Haleakalā) (3055 m in height). The minimal over-water distance between island sites is 162 km, and the ocean provides the presumed barrier. For three of the sites (Kahauale'a, Manukā and Makawao), all reciprocal pairwise crosses were attempted, to assess between island and within-island isolation. The Kīpahulu site provided a within-island comparison for Maui.

Since female spiders show a higher positional fixity on residence leaves, only females were transported between sites (henceforth, these transported females are referred to as "test females"). During transportation, spiders were held in 30 x 100 mm glass shell vials, provided with a section of hostplant leaf to provide a stable perch. The vials were stopped with wrapped cotton gauze to allow for respiration. An attempt was made to keep the temperature of the transported spiders below 20 degrees C and to maintain high humidity within the vials. The spiders were fed with small insects during their incarceration. At the new site, the spiders spent at least ten hours undisturbed in the vials in full shade at ground level, to acclimatize to the ambient temperature regime. The spiders were then released upon a suitable hostplant, and monitored closely as they settled upon the leaves.

Adult males from the allo-population were taken from a sample located out of the bounds of the study quadrat, and were required to fulfill criteria for maturity: 1) matching size category of males established by demographic studies (S. Gon III, thesis manuscript); 2) expressing morphological development of external genitalia and secondary sexual characteristics (pedipalpal development and pigmentation); 3) displaying typical courtship movements (described in Gon and Reese, in prep.) within three minutes of exposure to an iso-population female.

Starting from the day prior to testing, chosen males would be established on the same plants as test females. When nightly movements of males began at dusk, it was easy to direct the movements of males onto specific webs of test females without undue disturbance, by intercepting draglines of males performing web drops in exploratory movements, and placing the hanging males onto the leaf tops of test females. Observations began when males moved to the underside of a test female's leaf.

The behaviors observed and parameters recorded were as follows: 1) total time elapsed from courtship onset to end; 2) total counts for the two courtship Modal Action Patterns: pluck-wave and somatic bob; 3) responses of males to test female behaviors (*e.g.*, response to solicitation movements, or to agonistic behaviors).

#### Controls:

Test females were required to fulfill "behavioral normality" criteria at the new site, to control for the effect of transport: 1) maintenance of normal flattened motionless cryptic diurnal postures during the day, broken at night; 2) normal web-building/enhancement movements at dusk; 3) taking of offered live food items, or observation of natural prey capture; 4) positional fixity on a hostplant. If all of the above criteria were satisfied, the reactions of the test females to potential mates were recorded and included in the results.

To control for variability among the allo-populational males' courtship behaviors, each test female was exposed to two or three different males of the pool of males selected unless successful copulation occurred. Additionally, at the end of each male's testing, an iso-populational courtship was arranged to judge the variance in efficacy of the males' displays, and pairwise comparisons of allo and iso-populational performances were conducted.

To control for the effect of incarceration, the control (iso-populational) females used to test the acceptability of experimental males were incarcerated in identical glass shell vials as test females. They were maintained on offered live food for 24 hours and re-established on a new leaf of a new plant prior to being exposed to potential mates.

As a final control for the effect of transport and incarceration, test female spiders were returned to the original source sites and exposed to iso-populational males, and courtship acceptance was monitored and recorded. If courtship success was observed, this would be an indicator that transport and incarceration had not adversely affected courtship receptivity in those females. It became clear that test females returned to their source populations remained receptive to iso-populational courtship.

In order to avoid changing the genetic structure of the study populations, all test females were collected following completion of testing. If possible, for within-island tests, the females were returned to their source populations and released. For between-island tests, or if copulations were observed, females were collected and sacrificed to serve as voucher specimens for morphological comparisons between populations.

## RESULTS

The results are organized according to specific experiments involving pairs of study sites. Only information regarding success of courtship, the behaviors mediating courtship termination, and comparative courtship durations are presented in the narratives below. There were additional differences in the responses of males to allo-populational females, and these are presented in the tabular summary (Table 1). A matrix denoting the differences between iso-populational and allo-populational courtships is presented (Table 2).

### **INTER-ISLAND CROSSING EXPERIMENTS:**

#### **Makawao females moved to Kahauale'a**

Of 8 test females, 18 trials involving 9 males (one to three attempts each) were observed. All failed to culminate in copulation. No female solicitation movements were elicited. In 11 trials (7 males) courtship ended with the female chasing the male off web at courtship onset. In 6 trials (4 males) courtship ended when the female chased the male off the leaf a second time (after a first leaf top/webdrop evasion by male). One trial ended with the female vacating the leaf. The average test courtship duration was significantly shorter than the average Makawao iso-populational courtship duration (average test courtship duration  $3.93 \pm 3.15$  decimal minutes; average Makawao courtship duration  $8.71 \pm 2.34$  decimal minutes;  $t = 5.10$  significant at  $p < 0.005$ ).

#### **Makawao females moved to Manukā**

Of 16 test females, 26 trials involving 9 males (3 attempts each with one exception 2 attempts) were observed. All failed to culminate in copulation. No female solicitation movements were elicited. In 19 trials (7 males) the males were chased off the leaf at courtship onset. In the remaining 7 trials (3 males) the males were chased off the leaf after an initial web drop and resumption of courtship. The average test courtship duration was significantly shorter than the average Makawao iso-populational courtship duration (average test courtship duration  $4.31 \pm 2.69$  decimal minutes; average Makawao courtship duration  $8.71 \pm 2.34$  decimal minutes;  $t = 6.113$  significant at  $p < 0.005$ ).

#### **Kahauale'a females moved to Makawao**

Of the 8 test females, none met the normality criteria completely. Nonetheless, 10 attempts (5 males) were staged and observed. All failed to culminate in copulation. In 6 trials courtship ended when the female vacated the leaf by petiole walk. In the remaining 4 trials, courtship ended when the female web-dropped as the male approached.

### **Manukā females moved to Makawao**

Of 6 test females, none met the normality criteria completely. Nonetheless, 8 attempts (5 males) were staged and observed. All failed to culminate in copulation. In 3 trials courtship ended when the female vacated the leaf via petiole walk. In the remaining 5 trials, courtship ended when the female vacated the leaf by a web-drop exit to the next lower leaf.

### **WITHIN-ISLAND CROSSING EXPERIMENTS:**

#### **Kahauale'a females moved to Manukā**

Of 24 test females, 48 trials were observed involving 16 males (3 attempts each). All failed to culminate in copulation. No female solicitation movements were elicited. In 14 trials (7 males) the males were chased off the leaf at first courtship attempt. In 26 trials (10 males) males engaged in leaf top evasion and returned to a second courtship attempt, but were again attacked during the second courtship attempt. In the remaining 8 trials (5 males) courting stopped when the female vacated the leaf. Average allo-populational courtship duration was significantly shorter than average Kahauale'a iso-populational courtships (average test courtship duration  $2.30 \pm 2.11$  decimal minutes; average Kahauale'a courtship  $6.57 \pm 2.55$  decimal minutes;  $t = 5.307$   $p < 0.005$ ).

#### **Manukā females moved to Kahauale'a**

20 test females were presented with 13 males (3 trials for each male = 39 courtship attempts observed). All of the attempts failed to culminate in copulation. Of these, 6 trials (involving 4 males) ended with agonistic female behavior. 5 trials (2 males) ended when the female vacated the residence leaf. In the remaining 28 trials (11 males) courtship was "successful" in eliciting Manukā-specific female solicitation movements after significantly longer courtship attempt durations than Manukā iso-populational courtships (average allo-populational courtship duration  $9.88 \pm 5.44$  decimal minutes; average Manukā courtship duration  $7.46 \pm 1.41$  decimal minutes;  $t = 2.368$   $p < 0.05$ ). However, the Manukā solicitation movements drove all 7 males off (9 trials ended in male web drop by 3 males; in the remaining 19 trials, 4 males broke off courtship movements and retreated to the residence leaf top and exited via the petiole).

#### **Kīpahulu females moved to Waikamoi**

Of 6 test females, 18 attempts (6 males) were staged and observed. There were 10 failures, but also 8 copulations following solicitation movements. Of the failures, 6 trials (3 males) ended when the males retreated via leaf top evasion. In 2 trials (2 males) courtship ended when the female vacated the leaf via petiole walk, and in 2 trials (involving 1 male) the female vacated the

leaf via a web-drop. The average test courtship duration was not significantly different from the average Makawao iso-populational courtship duration (test courtship duration  $7.56 \pm 3.941$  decimal minutes; Makawao iso-populational courtship duration  $8.71 \pm 2.34$  decimal minutes;  $t$  value 0.960 is not significant at  $p > 0.1$ ). It is notable that this was the only interpopulational intra-island test that ended in copulations.

## DISCUSSION

### **Reproductive isolation justifies species revision**

It is clear that some of the allopatric populations of *Theridion grallator* are reproductively isolated; mediated by prezygotic, ethological barriers. This isolation is correlated with populational differences in morphology, demography and ecology. On these grounds a taxonomic revision of the single species into named, allopatric species is justified for these populations. It should be pointed out that despite their differences, the complex of species currently represented by the named taxon *Theridion grallator* comprise a natural group exhibiting unique defining characters relative to other endemic congeners.

### **Evolution of intrinsic isolation**

A dichotomy of viewpoints regarding the evolution of intrinsic isolating mechanisms focuses on the basis for the development of courtship divergence. In a classical view, the re-establishment of sympatry plays a large role in the erection of pre-zygotic barriers to reproduction (*e.g.*, Dobzhansky, 1970; Fisher, 1930,) and is an adaptational argument based on fitness deficits incurred by hybridization (for a discussion, see White, 1978; Bush, 1975; Kaneshiro, 1980). In another viewpoint (*e.g.*, see Muller 1939, 1942), evolution in allopatry culminates in divergent courtship patterns without invoking re-establishment of sympatry. These differences by themselves are sufficient to maintain genetic isolation should sympatry recur, but such a event is not necessary. Current thought regarding incipient speciation has tended to include stochastic rearrangements of adaptive gene complexes during founder events (*e.g.*, Carson, 1971; Templeton, 1980), and imply strongly that significant changes in the genotype and phenotype of organisms undergoing founder-type allopatric speciation are to be expected. Ethological traits are included in these changes (*e.g.*, Kaneshiro, 1976, 1980; Craddock, 1974; Spieth, 1981) the most significant of which would be courtship behaviors.

### **Asymmetries and the direction of evolution**

In Kaneshiro's (1980, 1983) discussion of courtship asymmetries, the idea that the direction of asymmetries would correlate with the ancestor-progeny relationships of populations was advanced (but see Moodie, 1982; Wasserman and Koepfer, 1980; Markow, 1981). Kaneshiro suggested that where there are

asymmetrical patterns of reciprocal courtship, the population in which females more frequently accept allo-populational male courtship is derived. The inference is dependent on the extent to which epigamic sexual selection plays a role in courtship success. It seems reasonable to conclude that courtship in *Theridion grallator* involves female acceptance of male courtship behaviors, with aggressive rejection being the modal basis for terminating unsuccessful courtships. The experiments with *Theridion grallator* provide the basis for a test of Kaneshiro's model with a taxon not previously considered. Moreover, morphological, ecological and behavioral data are available as independent measures of polarities (Gon, in prep.). On the basis of the courtship asymmetries alone, the Kahauale'a group is ancestral, and the Manukā group derived. This does not correspond with polarity judgments based upon demography and morphology, as the Kahauale'a population exhibits numerous unique characteristics. Perhaps the mechanism of "loss of behavioral elements" that Kaneshiro invokes for his theory does not apply for *Theridion grallator*. It may instead be that any such general changes in female acceptance or male courtship patterns become modified as each population evolves, and sexual selection proceeds in isolation. Because the reciprocal crosses between Maui and Hawai'i have not yet been completed, it is not yet possible to infer the direction of evolution between the Maui populations and the Big island populations. Those reciprocal crosses (Manukā females to Makawao and Kahauale'a females to Makawao) are therefore the next logical step in the study.

#### **Inter-island and within-island isolation**

On the basis of the results to date, it appears that inter-island isolation is stronger than within-island isolation. The success of Kahauale'a male courtship signals in eliciting Manukā female solicitation movements suggests that the courtship behaviors of the two groups are quite similar. The success of courtships culminating in copulations between the geographically distant Kīpahulu and Makawao populations on Maui likewise point to within-island maintenance of courtship patterns. Statistical analyses of male courtship behaviors and courtship durations provide additional corroboration of relative similarity. In contrast, inter-island courtships seem to be immediately aborted, and behavioral courtship elements show statistically significant differences. This provides strong evidence for the inference that between island barriers to gene flow are far less permeable than within-island barriers.

#### **Habitat heterogeneity and deme maintenance**

There is a great functional difference between the courtship signal success seen in the Manukā-Kahauale'a crossings and the courtship to copulations seen in the Kīpahulu-Makawao crossings. Only in the second case may we claim that the two populations are conspecific. There is a morphological similarity between the Kīpahulu and Makawao populations that correlates with their demonstrated courtship compatibility. This infers genetic similarity and

provides evidence that deme structure is maintained between the two populations. In light of the known low vagility of the spiders, the maintenance of the Maui deme across a distance of 22 km requires explanation. Why has deme structure been maintained on Maui, but not on the Hawai'i? In both cases the test populations were on opposite sides of imposing shield volcanoes that extend well above treeline (see Figure 1), but on Maui, morphological differences are low and courtship is compatible, while on Hawai'i, morphological differences are notable and reciprocal courtships were unsuccessful. The answer seems related to habitat heterogeneity.

Makawao and Kīpahulu represent near end-points in a broad, relatively homogenous band of mesophytic, *Metrosideros*-dominated forest that extends from the edge of the Kula grasslands, around through Makawao and Ko'olau, Ke'anae, Ha'ikū, Hāna, Kīpahulu and ending in the Manawainui Forest Reserve at Kaupō Gap. Representatives of *Theridion grallator* have been found at points along the band between the end-points, and at the end-points themselves. It seems clear now that the spiders maintain populations throughout the region and exchange genetic material across the region even if only via trivial movements of the individuals in their habitat. There are no major breaks in habitat for the spiders, and the stage is set for the broad distribution of a single species independent of poor vagility.

In contrast, the island of Hawai'i offers no such uninterrupted band of habitat between the Kahauale'a and Manukā populations. The vegetational systems of the two are different. In Kahauale'a the habitat is a *Metrosideros* kipuka in a geologically young rift-zone setting. In Manukā, the habitat is mixed mesophytic-dryland forest with higher overstory diversity. Between the two sites lies the Ka'ū acid cinder desert, a mosaic of grassland and agriculture, strips of relatively disturbed native forest, the South rift zone of Mauna Loa, and a vegetational shift into the Kona Forest system (Rock, 1913). It is somewhat surprising that the two populations showed such a high level of courtship compatibility. Nonetheless, it seems likely that the lack of an uninterrupted habitat band on Hawai'i has contributed to divergence of the populations there, and by contrast, the integrity of habitat on Maui has allowed for genetic homogeneity across the East Maui volcano forested flank.

It becomes an interesting conjecture to consider the effect of historical disturbance on *Theridion grallator*. It may be that until relatively recently, a smoother transition between the Kahauale'a site and the Manukā site existed, and that effective allopatry has been a post-disturbance phenomenon. Considering the relatively rapid potential rate of evolution among short-lived invertebrates, the spiders may owe their species status to such disturbance and the breakup of habitat that it entailed.



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### A NOTE ON THE SPELLING OF HAWAIIAN WORDS

The author has used two diacritical phonetic markings, the glottal stop (') and the macron (-), on all words of Hawaiian origin as appropriate (for example, Ka'ū, a district name). This is in accord with a 1978 recommendation on a uniform spelling system for the Hawaiian language.

**TABLE 1: SUMMARY OF QUANTIFICATIONS:****Part 1: Statistics of Normal Iso-populational Courtships at the Three Permanent Study Sites on Maui and Hawai'i:**

Site:	N	pluck-wave	somatic bob	time
Makawao	28.00	15.00 $\pm$ 3.27	24.68 $\pm$ 3.68	8.71 $\pm$ 2.34
Manukā	12.00	24.58 $\pm$ 7.13	24.58 $\pm$ 7.13	7.46 $\pm$ 1.41
Kahauale'a	13.00	10.31 $\pm$ 2.90	19.69 $\pm$ 3.25	6.57 $\pm$ 2.55

**Part 2: Statistics of Experimental Allo-populational Courtships Between Allopatric Populations of *Theridion grallator*:**

Experiment:	N	pluck-wave	somatic bob	time	fensol	copul
Maka-Kaha	18.00	6.33 $\pm$ 2.47	10.67 $\pm$ 3.98	3.93 $\pm$ 3.15	00.00	00.00
Maka-Manu	26.00	15.35 $\pm$ 2.28	15.35 $\pm$ 2.28	4.31 $\pm$ 2.69	00.00	00.00
Kipa-Maka	18.00	16.00 $\pm$ 3.82	25.50 $\pm$ 5.67	7.46 $\pm$ 3.94	44.44	44.44
Kaha-Manu	48.00	12.75 $\pm$ 5.88	12.75 $\pm$ 5.88	2.30 $\pm$ 2.11	00.00	00.00
Manu-Kaha	39.00	12.56 $\pm$ 5.26	23.31 $\pm$ 8.01	9.88 $\pm$ 5.44	71.79	00.00

Please refer to the figure legend for Table 1.

**TABLE 2: COMPARISON MATRICES OF NORMAL AND TEST COURTSIPS****Part 1: Courtship Durations:**

	MAKAWAO	sig?	MANUKA	sig?	KAHAUALA'A	sig?
Maka-Kaha	5.101	*****	3.880	*****	2.122	**
Maka-Manu	6.091	*****	4.535	*****	2.197	***
Kipa-Maka	1.115	NO	0.003	NO	0.624	NO
Kaha-Manu	12.831	*****	10.951	*****	5.307	*****
Manu-Kaha	1.091	NO	2.332	***	2.413	***

**Part 2: Frequency of pluck-wave MAP:**

Maka-Kaha	8.991	*****	3.789	*****	3.234	*****
Maka-Manu	0.418	NO	1.973	**	4.610	*****
Kipa-Maka	0.781	NO	1.672	*	3.681	*****
Kaha-Manu	1.829	**	2.328	***	1.633	*
Manu-Kaha	1.993	**	2.368	***	1.517	*

**Part 3: Frequency of somatic bob MAP:**

Maka-Kaha	9.843	*****	4.733	*****	5.152	*****
Maka-Manu	10.021	*****	1.973	**	3.450	*****
Kipa-Maka	0.451	NO	0.165	NO	2.702	****
Kaha-Manu	8.945	*****	2.328	***	4.177	*****
Manu-Kaha	0.776	NO	0.231	NO	1.726	**

Please refer to the figure legend for Table 2.

## FIGURE LEGENDS

**FIGURE 1:** The known localities of populations of *Theridion grallator* (dots) and the study sites (squares) occur between 1000 and 6000 ft. elev. For both Maui and Hawai'i, study sites are separated by shield volcanoes rising above 10,000 ft. It is notable that on Maui, habitat is unbroken between the study sites, while this is not so on Hawai'i (shaded areas represent relatively undisturbed forest systems within given altitudinal ranges). Habitat heterogeneity may explain the greater evolutionary divergence between Hawai'i populations vis-a-vis Maui populations.

**TABLE 1:** The table shows descriptive statistics (t values and significance levels) for the duration of courtships, and frequencies of the 2 major male courtship behaviors:

N = number of courtship trials observed; "pluck-wave" refers to one of two male courtship modal action patterns (MAPs); "somatic bob" is the second male courtship MAP; the statistics are the average frequency of MAPs per courtship attempt,  $\pm$  the standard deviation; "time" refers to the mean duration of courtship attempts in decimal minutes; "femsol" is the percentage of courtships which elicited the female copulatory solicitation MAP; finally, "copul" refers to the percentage of courtship trials that culminated in copulation. The population names are abbreviated as follows: Kaha=Kahauale'a (Puna, Hawai'i); Manu=Manukā (Kau, Hawai'i); Kipa=Kīpahulu (Maui); Maka=Makawao (Maui). Where names are paired, the first refers to the source population for test females, and the second refers to the population into which test females were transported (e.g., Kipa-Manu=Kīpahulu females transported to Makawao and exposed to Makawao male courtship).

**TABLE 2:** When the information in Table 1 is used in a t-test for means, a comparison of courtship durations and male MAPs in iso-population (control) vs. allo-population (experimental) courtships yields 3 matrices of t values. Each matrix describes the results from all pair-wise comparisons for each variable (e.g., the variable "frequency of pluck-wave per courtship"). Asteriks indicate the level at which the t values are significant, according to this key:

***** = significant at $p < 0.005$	** = significant at $p < 0.050$
**** = significant at $p < 0.010$	* = significant at $p < 0.100$
*** = significant at $p < 0.025$	NO = $p > 0.100$ not significant.

These t values are useful in demonstrating differences between groups, and in identifying areas of divergence. For example, because the Kīpahulu-Makawao crosses were successful (ended in copulation) we expect no significant differences in characteristics of male courtship behavior between the Makawao group and the Kīpahulu group. We see that at all Makawao/Kipa-Maka intersections in all three parts of Table 2, there are no significant differences. Additionally, an examination of the Manu-Kaha vs Kaha-Manu rows indicates the asymmetry in courtship behaviors that yield asymmetrical courtship success in the field.

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# FIGURE 1: Study sites and Spider Habitat

